Clin. exp. Immunol. (1982) **48**, 353–358.

A complete selective C1q deficiency in a patient with discoid lupus erythematosus (DLE)

AKIKO UENAKA,† T. AKIMOTO, T. AOKI, I. TSUYUGUCHI & K. NAGAKI*
*The Osaka Prefectural Habikino Hospital and * The Research Institute, The Centre for Adult Diseases, Osaka, Japan*

(Accepted for publication 27 November 1981)

SUMMARY

A 32 year old male patient with discoid lupus erythematosus (DLE) was found to have a complete, selective deficiency of C1q subcomponent of the complement assessed either by haemolytic assay or by protein determination. Addition of highly purified C1q completely restored the complement haemolytic activity of the patient's serum. Neither C1q precipitin nor anti-complementary activity was detected. Lymphocytes isolated from the patient's peripheral blood, however, bound as many C1q molecules as those of healthy control individuals. The patient is in good health except for skin lesions. A low level of circulating immune complexes was detected in his serum, but no C1q molecules were bound. Serum complement activities of the patient's mother and two other siblings were within the normal range. An impaired synthesis of C1q protein was strongly suggested as the cause of C1q deficiency in this patient's serum.

INTRODUCTION

C1q, a subcomponent of the complement, is capable of binding immune complexes and thereby initiating the activation of the classical pathway's cascade of the complement system. Many studies have been reported on the deficiencies of complement components, C1 through C9, but very few reports on selective complete C1q deficiency have been described so far (Berkel *et al.*, 1977; Leyva-Cobian *et al.*, 1981).

In this communication, a study was performed on a complete lack of serum C1q subcomponent in a 32 year old man who was otherwise healthy except for a skin lesion compatible with discoid lupus erythematosus (DLE).

CASE HISTORY

A 32 year old man visited the hospital because of a skin rash. He was well until 21 years old, when drug-induced cheilitis developed after taking a medicine for colds. Years before the visit, he noticed an itchy skin rash on his upper limbs and shoulder which was gradually spread. He had been otherwise healthy and working as an electrician for 15 years. His father suffered from rheumatoid arthritis but his mother, two brothers and two sisters are in good health.

On examination the patient appeared well. Scattered erythematous and whitish erosive skin lesions were seen in his upper limbs and face, other vital signs were normal. Laboratory data revealed a moderate leucopenia, normal erythrocyte sedimentation rate and normal urinalysis. L-E

† Present address and correspondence: The Research Institute, The Centre for Adult Diseases, 3 Nakamichi 1-chome, Higashinari-ku, Osaka 537, Japan.

0009-9104/82/0500-0353$02.00 © 1982 Blackwell Scientific Publications

cell preparations, antinuclear antibody and rhematoid factor were negative. Automated chemical analysis of his serum showed no abnormality. Serum complement activity was below 3·0 units/ml (Pooled normal human serum; 45 CH50 units/ml). The histological examination of a skin biopsy obtained from the left forearm established the diagnosis of DLE. The skin lesions responded temporarily to steroid therapy.

MATERIALS AND METHODS

Serum and plasma. Blood was allowed to clot for 90 min at 37°C and centrifuged for 10 min at 3,000 rpm. EDTA (ethylene diamine-tetra-acetic acid) plasma was obtained from 2 ml of venous blood drawn into plastic tube containing 3 mg EDTA 3 Na. The serum and plasma were kept frozen in small aliquots at −80°C until use.

Functional assay of complement and its components. Haemolytic activity of complement was measured according to the method of Mayer, Kabat & Mayer (1961). For the sensitization of sheep erythrocytes a 1:600 or a 1:800 dilution of rabbit antiserum was used, as described by Mayer *et al.* (1961); this was the amount sufficient to produce 50% lysis in the presence of a 1:500 dilution of guinea-pig serum. C1 haemolytic activity was measured by the method of Borsos & Rapp (1963). The activities of the other complement components were measured using appropriate intermediate cells and reagents (Inai *et al.*, 1964; Nagaki, Iida & Inai, 1974; Iida *et al.*, 1976). C1q haemolytic activity was measured by the method of Kolb with slight modifications (Kolb, Kolb & Podack, 1979). Haemolytic complement activity through the alternative pathway was measured by the modified method of Platts-Mills & Ishizaka (1973) using rabbit erythrocytes. Complement activity of the serum was also assayed by the immune-adherence haemagglutination test (Nishioka, 1963).

Protein analysis. Highly purified C1q (human) was obtained according to the method described by Yonemasu & Stroud (1971). The protein concentration of C3, C4 and C1-inactivator in the serum were estimated using immunodiffusion plates (Partigen, Behringwerke AG, Germany). For the estimation of the protein concentration of C1q, the immunodiffusion plates were prepared using monospecific rabbit anti-C1q antiserum. Monospecific anti-C1r antiserum and anti-C1s antiserum were raised in rabbits by immunizing with the purified C1r or C1s, respectively.

Other immunological studies. Immune complexes were detected by the polyethylene glycol (PEG) precipitation complement consumption method described by Harkis & Brown (1979). C1q incorporated into the immune complexes was analysed by the double diffusion method using solubilized 2·5% PEG precipitates (precipitated without EDTA). The ability of the patient's serum to precipitate C1q was assayed by the method of Agnello, Winchester & Kunkel (1970). To detect anti-complementary activity in the patient's serum, the mixture of patient's serum and pooled human serum was incubated for 30 min at 37°C and the complement activity of the reaction mixture was measured.

Rosette assay. Peripheral blood lymphocytes were isolated from the heparinized venous blood by the Ficoll-Hypaque sedimentation technique. A rosetting method was employed for the determination of C1q-receptor bearing cells utilizing C1q coupled bovine erythrocytes as indicator cells (Gabey *et al.*, 1979). Briefly, after 16 hr incubation in 2% glutaraldehyde, bovine erythrocytes were extensively washed and incubated for 30 min at 37°C in 10 volumes of phosphate-buffered saline (PBS; pH 7·4) containing L-lysin (10 mg/ml), followed by the incubation with purified C1q (200 μg/ml) at 22°C. After 45 min, the cells were washed and used as the indicator cells. The indicator cells were incubated with peripheral blood lymphocytes for 5 min at 37°C, followed by centrifugation for 5 min at 600 r.p.m. The erythrocyte–lymphocyte mixtures were incubated in an ice bath for 30 min. Cells suspended in eosin-Y dye were examined and viable mononuclear cells binding at least three indicator cells were counted as rosette forming or C1q-receptor bearing cells. As a control, rosette formation was performed with glutaraldehyde treated bovine erythrocytes.

RESULTS

Complement profile

The complement components profile of the patient's serum was summarized in Table 1. The whole

Table 1. Complement profile of the case (T.N.)

	Haemolytic activity*		Protein concentration (mg/dl)		
	T.N.	NHS†		T.N.	normal range
Whole complement (CH50)	<3‡	45	C1q	undetectable	7–19
C1q plasma	undetectable	—	C4	34	15–45
C1q serum	undetectable	12,000	C3	72	60–110
C1	7·5	130,000	C1-INA(%)§	140	100
C2	2,000	1,900			
C4	65,570	50,630			
C3–C9	2,340	2,140			

* Expressed as site forming units/ml except for CH50 and C3–C9. CH50 and C3–C9 were expressed as units/ml

† NHS = Pooled normal human serum

‡ Haemolytic activity could not be detected in six experiments but in only one time activity was 2·7 units/ml

§ C1-INA = C1 inactivator. Expressed as % of the level of pooled normal human serum

complement activity was lower than 3·0 units/ml (normal pooled serum; 45·0 units/ml) and C1 activity was 7·5 SFU/ml versus 130,000 SFU/ml in pooled normal serum. The levels of C2, C4, C3–C9 activities and C4, C3 and C1-inactivator protein levels were within normal range. C1q protein was undetectable by the method of radial immunodiffusion analysis. And also C1q haemolytic activity was not detected in EDTA plasma and serum. On double diffusion analysis (Fig. 1), patient's serum formed precipitin lines against anti-C1r and against anti-C1s but no line was formed against anti-C1q. Haemolytic activity of the alternative complement system was within normal range. The whole complement activity and C1 activity in the patient's serum remained almost zero throughout the investigation period from June 1979. Family studies were performed on his mother and two brothers. Complement haemolytic activities and C1 activities (or C1q protein) in their sera were within normal range (Table 2). No further examination of the family was possible.

Immune-adherence haemagglutination activity

Complement bound antigen–antibody complexes are known to attach to primate erythrocytes (Nelson *et al.*, 1953). The sensitivity of this method for the titration of human serum complement has been reported to exceed that of the immune haemolysis. The early acting components, C1, C4, C2 and C3, were shown to be prerequisite for this phenomenon (Nishioka, 1963). As shown in Table 3, the titre of the patient's serum was as low as 1:100, compared with that of 1:4,000 in pooled normal human serum. The heat inactivation or chelation of Ca^{++} and Mg^{++} with 10 mM EDTA completely reduced the haemagglutination activity in either the patient's serum or pooled normal human serum.

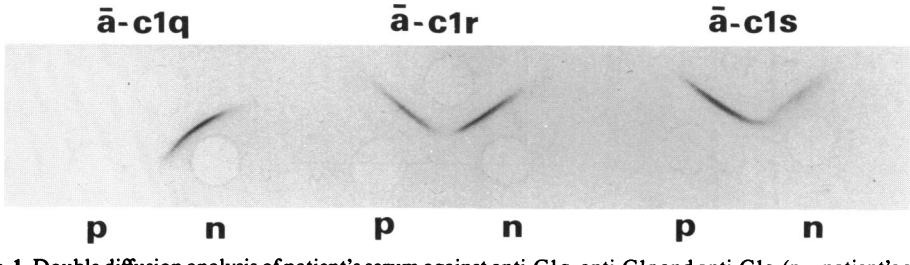

Fig. 1. Double diffusion analysis of patient's serum against anti-C1q, anti-C1r and anti-C1s. (p = patient's serum and n = normal human serum).

Table 2. Complement activity of the patient's family

	CH50†	C1 activity‡	C1q protein‡
Patient	<3·0	0·006	not detected
Mother	46·0	n.d.	92·5
Brother I	36·0	109·0	85·2
Brother II	41·0	112·1	92·5
NHS*	37·0	100·0	100·0

* Pooled normal human serum
† Expressed as units/ml
‡ C1 activity and C1q protein were expressed as % of the level of pooled normal human serum
n.d. = Not done

Table 3. Immune adherence patterns

	Serial dilutions of serum								
	1:50	1:100	1:250	1:500	1:1,000	1:2,000	1:4,000	1:6,000	1:8,000
T.N.	3	2	1	0	0	0	0	0	0
NHS	4	4	4	4	4	4	3	1	0
T.N. (56°C, 30 min)	0	0	0	0	0	0	0	0	0
NHS (56°C, 30 min)	0	0	0	0	0	0	0	0	0
T.N.—10 mM EDTA	0	0	0	0	0	0	0	0	0
NHS—10 mM EDTA	0	0	0	0	0	0	0	0	0

Mixtures of serially diluted serum (0·8 ml) and 0·1 ml of EA (1×10^8/ml) were incubated for 30 min at 30°C. And then 0·1 ml of human erythrocytes (O-type, 2×10^8/ml) were added to these mixtures and incubated for 10 min with shaking and left settled for another 60 min at 37°C. Haemagglutination pattern was read from 0 (no agglutination) to 4 (agglutinated strongly).

Restoration of C1 activity by purified human C1q

Increasing doses of C1q preparation were added to the patient's serum. After incubation for 30 min at 37°C, C1 activity in the patient's serum was assayed. As shown in Fig. 2, the addition of C1q restored the C1 activity of the patient's serum in a dose-response fashion. Whole complement activity of 39·0 units/ml was obtained when 25 μg of C1q were added to 1 ml of the patient's serum,

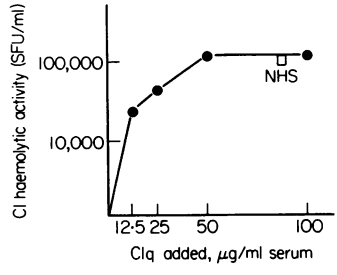

Fig. 2. Restoration of the C1 activity by purified human C1q. The purified C1q was added to the patient's serum and incubated for 30 min at 37°C, and the C1 activities were assayed. Haemolytic activities were expressed as site forming units/ml. □ = Pooled normal serum.

and complete restoration of C1 activity (130,000 SFU/ml) was obtained by the addition of 50 μg of C1q/ml serum. Because the C1q concentration in normal pooled serum was 70–190 μg/ml, these results indicate the complete absence of C1q in the patient's serum.

C1q precipitin activity, anti-complementary activity and immune complexes

Agnello *et al.* (1971) reported on the presence of C1q precipitins reactive with C1q in the serum of patients who showed low serum complement haemolytic activity. But, by the gel diffusion method, a precipitin line was not demonstrated between the patient's serum and purified C1q. Also the addition of increasing amounts of patient's serum did not reduce the complement activity in pooled normal serum, indicating the absence of anti-complementary activity in the patient's serum. The amount of complement consumed by the PEG precipitate obtained from the patient's serum was 1·8 times greater than that of the healthy adults which consumed very small amounts of complement. Further studies were performed to determine whether these immune complexes bore C1q-molecules. But solubilized PEG precipitates did not form a precipitin line against anti-C1q.

Detection of C1q-receptor bearing lymphocyte

The enumeration of C1q-receptor bearing lymphocytes among the patient's peripheral blood lymphocytes were performed by the rosette forming method with C1q coupled bovine erythrocytes. The average value of rosette forming cells among peripheral blood lymphocytes from normal healthy adults ($n=8$) was $10{\cdot}9 \pm 1{\cdot}0\%$ and that of the patient was 11·8%.

DISCUSSION

This patient is a case of discoid lupus erythematosus with complete deficiency of C1q.

Low serum level of C1q were observed in patients with Swiss type agammaglobulinemia (Gleich & McDuffie, 1966) and in patients with X-linked type agammaglobulinemia (Kohler & Müller-Eberhard, 1972). In these patients, increased catabolic rate of C1q was reported (Kohler & Müller-Eberhard, 1969). Marked reductions of C1q were also reported in patients with systemic lupus erythematosus (SLE) and in hypocomplementemic vasculitis-urticaria syndrome (HVUS) (McDuffie *et al.*, 1973; Sissons, Williams & Peters, 1974; Marder *et al.*, 1976, 1978). The C1q molecule is capable of binding antigen–antibody complexes and low levels of C1q observed in patients with SLE have been attributed to the consumption of C1q molecules by circulating immune complexes, and similarly consumption of C1q by C1q precipitins might be a cause of the reduction of C1q level in patients with HVUS. In SLE and HVUS patients, C4 and C2 levels were also low.

In contrast with these reports, the patient reported here had normal serum levels of immunoglobulins, his C4 and C2 levels were normal, and C1q precipitins were not demonstrated. Although circulating immune complexes, which consumed very small amounts of complement, were demonstrated by the PEG precipitation method, C1q molecules were not detected in the precipitates by the immunodiffusion method. In addition, L-E cell preparations and L-E tests were negative and rheumatoid factor and antinuclear factor were not elicited throughout the study period. These results strongly suggest that impaired synthesis of C1q is a causative mechanism of C1q deficiency in this case, and that the consumption of C1q by the immune complexes is unlikely.

A complete deficiency of C1q activity was found in a boy with glomerulonephritis (Thompson *et al.*, 1980), but antigenically C1q-related protein was present in his serum. The first case of a complete C1q protein deficiency was found in a 10 year old boy (Berkel *et al.*, 1977). He suffered from recurrent skin infections since childhood and died of sepsis. Light and electron microscopic findings of his kidney were compatible with a diagnosis of mesangioproliferative glomerulonephritis. Recently, Leyva-Covian *et al.* (1981) reported a familial C1q deficiency in three siblings with Rothmund–Thomson syndrome and mesangial proliferative glomerulonephritis with diffuse IgM deposits. But the present case, is now 32 years old, and has not suffered from either infectious diseases or renal diseases, doing daily work as an electrician without any physical complaints and enjoying good health except for skin lesions. C1q protein in the sera of his mother and two siblings

were normal. It remains to be elucidated whether there exists any causative relation between the C1q deficiency and DLE.

The writers wish to thank Professor Shinya Inai, Osaka Medical College, for his valuable advice throughout the study and Dr Kazuo Yamamoto, the president of Habikino Hospital, for his critical review of this manuscript.

REFERENCES

AGNELLO, V., WINCHESTER, R.J. & KUNKEL, H.G. (1970) Precipitin reactions of the C1q component of complement with aggregated γ-globulin and immune complexes in gel diffusion. *Immunology*, **19,** 909.

AGNELLO, V., KOFFLER, D., EISENBERG, J.W., WINCHESTER, R.J. & KUNKEL, H.G. (1971) C1q precipitins in the sera of patients with systemic lupus erythematosus and other hypocomplementemic states: characterization of high and low molecular weight types. *J. exp. Med.* **134,** 228s.

BERKEL, A.I., SANAL, Ö., THESON, R. & LOOS, M. (1977) A case of selective C1q deficiency. *Turkish J. Pediatrics*, **19,** 101.

BORSOS, T. & RAPP, H.J. (1963) Chromatographic separation of the first component of complement and its assay on a molecular basis. *J. Immunol.* **91,** 851.

GABEY, Y., PERLMANN, H., PERLMANN, P. & SOBEL, A.T. (1979) A rosette assay for the determination of C1q receptor-bearing cells. *Eur. J. Immunol.* **9,** 797.

GLEICH, G.J. & MACDUFFIE, F.J. (1966) Swiss type agammaglobulinemia associated with an abnormality of inflammatory response. *J. Pediatics*, **69,** 981.

HARKIS, G.D. & BROWN, D.L. (1979) Detection of immune complexes by a new assay, the polyethylene glycol precipitation-complement consumption test (PEG-CC). *Clin. exp. Immunol.* **36,** 117.

IIDA, K., FUJITA, T., INAI, S., SASAKI, M., KATO, T. & KOBAYASHI, K. (1976) Complement fixing abilities of IgA myeloma proteins and their fragments: The activation of complement through the classical pathway. *Immunochemistry*, **13,** 747.

INAI, S., FUJIKAWA, K., TAKAHASHI, H. & NAGAKI, K. (1964) Studies of the fourth component of complement. 1. Titration of the fourth component of complement in human serum. *Biken J.* **6,** 237.

KOHLER, P.F. & MÜLLER-EBERHARD, H.J. (1969) Complement–immunoglobulin relation: deficiency of C1q associated impaired immunoglobulin G synthesis. *Science*, **163,** 474.

KOHLER, P.F. & MÜLLER-EBERHARD, H.J. (1972) Metabolism of C1q. Studies in hypogammaglobulinemia, myeloma and systemic lupus erythematosus. *J. clin. Invest.* **51,** 868.

KOLB, W.P., KOLB, L.M. & PODACK, E.R. (1979) C1q: Isolation from human serum in high yield by affinity chronatography and development of a highly sensitive hemolytic assay. *J. Immunol.* **122,** 2103.

LEYVA-COBIAN, F., MONEO, I., MAMPASO, F., SANCHEZ-BAYLE, M., ECIJA, J.L. & BOOTELLO, A. (1981) Familial C1q-deficiency associated with renal and cutaneous disease. *Clin. exp. Immunol.* **44,** 173.

MARDER, R.J., RENT, R., CHOI, E.Y.C. & GEWURZ, H. (1976) C1q deficiency associated with urticarial-like lesions and cutaneous vasculitis. *Am. J. med.* **61,** 560.

MARDER, R.J., BURCH, F.X., SCHMID, F.R., ZEISS, C.R. & GEWURZ, H. (1978) Low molecular weight C1q-precipitins in hypocomplementemic vasculitis-urticaria syndrome: partial purification and characterization as immunoglobulin. *J. Immunol.* **121,** 613.

MAYER, M.M., KABAT, E.A. & MAYER, M.M. (1961) Complement and complement fixation. In *Experimental Immunochemistry*. 2nd edn. p. 133. Charles C. Thomas, Springfield.

MCDUFFIE, F.C., SAMS, W.M., JR., MALDONADO, J.E., SNDREINI, P.H., CONN, D.L. & SAMAYDA, E.A. (1973) Hypocomplementemia with cutaneous vasculitis and arthritis. Possible immune complex syndrome. *Mayo clin. Proc.* **48,** 340.

NAGAKI, K., IIDA, K. & INAI, S. (1974) A new method for the preparation of EAC14 cell with human or guinea-pig serum. *J. Immunol. Methods*, **5,** 307.

NELSON, R.A. (1953) The immune-adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. *Science*, **118,** 733.

NISHIOKA, K. (1963) Measurements of complement by agglutination of human erythrocytes reacting in immune-adherence. *J. Immunol.* **90,** 86.

PLATTS-MILLS, T.A.E. & ISHIZAKA, K. (1973) Activation of the alternative pathway of human complement by rabbit cells. *J. Immunol.* **113,** 348.

SISSONS, J.G.P., WILLIAMS, D.G. & PETERS, D.K. (1974) Skin lesions, angioedema and hypocomplementemia. *Lancet*, **ii,** 1350.

THOMPSON, R.A., HAENEY, M., REID, K.B.M., DAVIS, J.G., WHITE, R.H.R. & GAMERRON, A.H. (1980) A genetic defect of the C1q subcomponent of complement associated with childfood (immune complex) nephritis. *New Engl. J. Med.* **303,** 22.

YONEMASU, K. & STROUD, R.M. (1971) C1q: Rapid purification method for preparation of monospecific antisera and for biochemical studies. *J. Immunol.* **106,** 304.